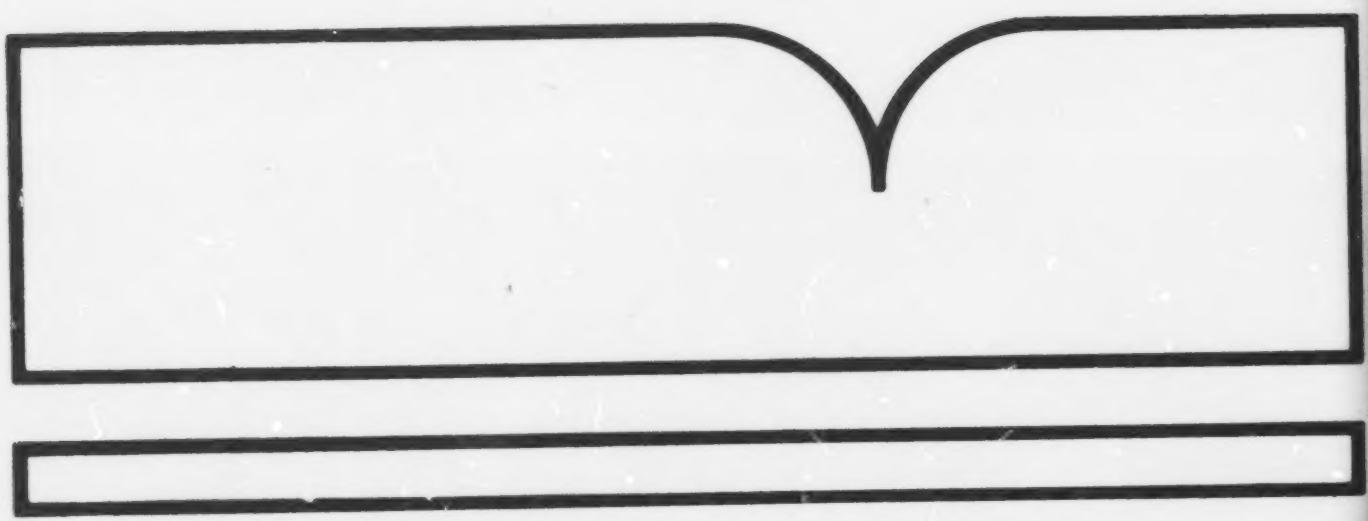
PB90-126046

# Toxicological Methods for Determining the Effects of Contaminated Sediment on Marine Organisms

(U.S.) Environmental Research Lab., Narragansett, RI

1984



U.S. Department of Commerce
National Technical Information Service
NTIS

**TECHNICAL REPORT DATA**
*(Please read Instructions on the reverse before completing)*

| 1. REPORT NO. | 2. | 3. PB90-126046 |
|---|---|---|
| EPA/600/J-84/427 | | |

**4. TITLE AND SUBTITLE**
TOXICOLOGICAL METHODS FOR DETERMINING THE EFFECTS OF CONTAMINATED SEDIMENT ON MARINE ORGANISMS

**5. REPORT DATE**
accepted 1984

**6. PERFORMING ORGANIZATION CODE**

**7. AUTHOR(S)**
Richard C. Swartz

**8. PERFORMING ORGANIZATION REPORT NO.**
ERLN-N056

**9. PERFORMING ORGANIZATION NAME AND ADDRESS**
US EPA, Office of Toxic Substances
North Texas State University

**10. PROGRAM ELEMENT NO.**
B101

**11. CONTRACT/GRANT NO.**

**12. SPONSORING AGENCY NAME AND ADDRESS**
US EPA, Environmental Research Laboratory
South Ferry Road
Narragansett, R.I. 02882-1198

**13. TYPE OF REPORT AND PERIOD COVERED**
Journal article

**14. SPONSORING AGENCY CODE**
EPA 600/05

**15. SUPPLEMENTARY NOTES**
Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems pp.183-198 (1984)

**16. ABSTRACT**

Most chemicals and waste materials discharged into the marine environment contain fractions that eventually accumulate in sediment. Uncertainty about the ecological significance of sediment contamination to benthic, epibenthic, and pelagic communities has prompted development of methods for testing sediment toxicity. The Environmental Protection Agency (EPA)/Army Corps of Engineers (USACE) Implementation Manual (1), mandated by the 1977 Ocean Dumping Regulations, provided the first guidance for sediment bioassays. During the next 7 years, a great variety of ecotoxicological methods were used to evaluate sediment contamination. This paper reviews these methods and evaluates their application in regulatory, monitoring, and research programs.

**17. KEY WORDS AND DOCUMENT ANALYSIS**

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| sediment toxicity<br>bioaccumulation<br>experimental design<br>marine organisms<br>bioassay | | |

| 18. DISTRIBUTION STATEMENT | 19. SECURITY CLASS *(This Report)* | 21. NO. OF PAGES |
|---|---|---|
| RELEASE TO PUBLIC | UNCLASSIFIED | 18 |
| | 20. SECURITY CLASS *(This page)* UNCLASSIFIED | 22. PRICE |

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL INFORMATION SERVICE
SPRINGFIELD, VA. 22161

EPA/600/J-84/422

Chapter 14

# Toxicological Methods for Determining the Effects of Contaminated Sediment on Marine Organisms

Richard C. Swartz

NTIS is authorized to reproduce and sell this report. Permission for further reproduction must be obtained from the copyright owner.

## INTRODUCTION

Most chemicals and waste materials discharged into the marine environment contain fractions that eventually accumulate in sediment. Uncertainty about the ecological significance of sediment contamination to benthic, epibenthic, and pelagic communities has prompted development of methods for testing sediment toxicity. The Environmental Protection Agency (EPA)/Army Corps of Engineers (USACE) Implementation Manual (1), mandated by the 1977 Ocean Dumping Regulations, provided the first guidance for sediment bioassays. During the next 7 years, a great variety of ecotoxicological methods were used to evaluate sediment contamination. This paper reviews these methods and evaluates their application in regulatory, monitoring, and research programs.

## OVERVIEW

Representative methods for determining the toxicity of sediment to marine organisms are summarized in Table 14.1. Most of these techniques were not developed specifically for use in permit programs of regulatory agencies. Response criteria range from biochemical through community levels of biological organization. With some exceptions, the response criteria fall into four groups: larval development and survival, behavior (especially sediment

Table 14.1 Bioassay Methods for Determining the Effects of Contaminated Sediment on Marine Organisms

| Response criterion | Species | Collection site of tested sediment; or toxic material added to control sediment | Sediment phase[a] | Reference |
|---|---|---|---|---|
| Detoxifying enzyme induction | Polychaete, *Capitella capitata* | benz(a)anthracene | So, 2000 g, FT, 10 wk | (2) |
| Bioluminescence inhibition | Bacterium, *Photobacterium phosphoreum* | crude oil<br>Puget Sound | Li, St, 15 min | (56) |
| Chromosome damage | Fish, *Salmo gairdnerii* | Puget Sound | Li, St, 3 d | (3, 57) |
| Egg/larval development | Fish, *Hypomesus pretiosus* | Puget Sound | Su, Li, 80 g, 35 d + | (4) |
| Larval development | Bivalve, *Crassostrea gigas* | Puget Sound | Su, 15 g,St, 2 d | (5) |
| Larval physiology, | Shrimp, *Palaemonetes pugio;* | Charleston Harbor | Su, Li, St, 1 min- 30 d | (6) |
| Larval mortality | polychaete, *Polydora sp.*<br>Fishes, *Brevoortia tyrranus, Lagodon rhomboides, Paralichthys dentatus, P. albigutta, P. lethostigma, Leiostomus xanthurus, Micropogon undulatus* | Charleston Harbor | Li, St, 4 d | (7) |
| Respiration | Oligochaeta, *Monopylephorus cuticulatus* | Puget Sound | So, Li, St, 8 h | (3) |
| Osmoregulation | Fish, *Oncorhynchus kisutch* | Grays Harbor | Su, FT, 9 d | (8) |
| Sediment avoidance | Echinoderms, *Brisaster latifrons Chiridota laevis;* squat-lobster, *Munida quadrispina;* crabs, *Cancer magister, Hemigrapsus oregonesis, Chionoecetes bairdi;* shrimps, *Crangon alaskensis, Pandalus danae;* bivalve, *Macoma inconspicua;* amphipod, *Corophium slamonis* | NW Canadian coast | So, St, 1-3 d | (9) |

Table 14.1 (contd.)

| Response criterion | Species | Collection site of tested sediment; or toxic material added to control sediment | Sediment phase[a] | Reference |
|---|---|---|---|---|
| Sediment avoidance | Amphipod, *Rhepoxynuis* spp | sewage, Zn, Cd,<br>Palos Verdes Shelf | So, Ft, 3 d | (10) |
| Sediment avoidance | Crab, *Carcinus maenas;* shrimp, *Crangon crangon;* fishes, *Solea solea, Pomatoschistus minutus* | parathion, malathion | So, 3.3 L, FT, 10 min | (11) |
| Burrowing behavior | Fish, *Ammodytes hexapterus* | crude oil | So, Ft, 4 d | (12) |
| Burrowing behavior | Bivalve, *Mercenaria mercenaria* | crude oil | So, 40 L, FT, 4 d | (13) |
| Burrowing behavior | Bivalve, *Protothaca staminea* | Cu | So, 3.3-7.1 L, FT, 2 d + | (14) |
| Burrowing behavior | Bivalves, *Cerastoderma edule, Abra alba, Macoma baltica;* polychaetes, *Nereis diversicolor, Scoloplos armiger* | parathion, malathion | So, 3.3 L, FT, 20 h | (11) |
| Feeding activity | Polychaete, *Arenicola cristata* | kepone | So, 62 L, St, 6 d | (15) |
| Predator avoidance | Bivalve, *Protothaca staminea* | crude oil | So, 135 L, Fe, 13-29 d | (16) |
| Reproduction, mortality | Mysid shrimp, *Mysidopsis bahia* | East Bay | Su, FT, 4-28 d | (17) |
| Pathology, mortality | Fish, *Leiostomus xanthurus* | Elizabeth River | So, FT, 8 d | (58) |
| Acute mortality | Amphipod, *Rhepoxynius abronius* bivalves, *Protothaca staminea, Macoma inquinata;* Polychaete, *Glycinde picta;* Cumacea | Puget Sound<br>Raritan River<br>Houston Ship Channel | So, 1.8 L, FT, 10 d | (18) |
| Acute mortality | Amphipod, *Rhepoxynius abronius* | Commencement Bay, WA<br>Palos Verdes Shelf<br>sewage sludge, CD | So, 0.18 L, St, 10 d | (19, 20, 21, 22, 59, 60) |
| Acute mortality | Fishes, *Fundulus heteroclitus, Leiostomus xanthurus;* bivalve, *Mya arenaria* | Baltimore Harbor | Su, St, 2 d | (23) |

Table 14.1 (Contd.)

| Response criterion | Species | Collection site of tested sediment; or toxic material added to control sediment | Sediment phase[a] | Reference |
|---|---|---|---|---|
| Acute mortality | Fishes, *Menidia menidia, Cyprinodon variegatus, Fundulus similis* | New York Harbor | So, Su, 3.5 L, FT, 52 d | (24) |
| Acute mortality | Shrimp, *Crangon septemspinosa;* polychaete, *Nereis virens* | endrin, endosulfan, dieldrin, chlordane DDT, PCB, HCB | So, 500 g, St, 4-12 d | (25, 26) |
| Acute mortality | Shrimp, *Palaemonetes pugio* | Hampton Roads<br>Duwamish River, WA<br>Los Angeles Harbor | So, Su, Li, St, 4-10 d | (27, 28) |
| Acute mortality | Copepods, *Acartia tonsa, Tigriopus californicus*; shrimps, *Palaemonetes pugio, P. vulgaris,* Mysidopsis bahia; amphipod, *Parahoustorius* sp; isopod; *Sphaeroma quadridentatum* | Duwamish River, New York Harbor, Long Island Sound | So, Su, LI, St, 4-15 d | (29) |
| Acute mortality, pathology | Amphipod, *Ampelisca abdita* | Cu, As | metals in seawater, 4-6 d | (30) |
| Mortality | Shrimp, *Crangon* spp; crab, *Cancer magister*; mussel, *Mytilus californianus*; tunicate, *Ascidia ceratodes*; lobster, *Homarus americanus* | San Francisco Bay | Su, FT, 3 weeks | (31) |
| Population growth | Nematodes, *Chromadorina germanica, Diplolaimella punicea* | New York Bight | So, 0.06 L, St, 2 wk | (32) |
| Full life cycle | Polychaete, *Capitella capitata* | Puget Sound | So, Su, Li, St, 35 d + | (4, 61) |
| Community recolonization | Macrobenthos | sewage sludge | So, 6.7 L, Fe, 4 wk | (33) |



Table 14.1 (Contd.)

| Response criterion | Species | Collection site of tested sediment; or toxic material added to control sediment | Sediment phase[a] | Reference |
|---|---|---|---|---|
| Community recolonization | Macrobenthos | barite; PCB | So, 3 L, FT, 10 wk | (34, 35, 36) |
| Community recolonization | Macrobenthos | crude oil | So, 6.8 L, FE, 15 mo | (37) |
| Community/population density | Macrobenthos | sewage sludge | So, 4 $m^2$ plots, FE, 8 mo | (38) |
| Community structure | Macrobenthos | sewage sludge | So, 1.2 $m^2$ plots, FE, 12 mo | (39) |
| Microcosm structure/function | Macrobenthos | phthalate ester | So, FT, 30 d | (40) |
| Microcosm structure/function | Macrobenthos and meiobenthos | fuel oil, oil mousse; Narragansett Bay | So, 800 L +, FT, 7 mo + | (41, 42, 43, 44, 45) |

[a] So = Solid; Su = Suspended; Li = Liquid Sediment Mass (L or g); Fe = Field Exposure; Seawater Supply: St = Static; FT = Flow-through; Duration

avoidance and burrowing), acute mortality, and community structure or function. Effects on biochemistry, genotoxicity, physiology, and population dynamics have been examined less frequently. A great variety of fish and benthic invertebrate species have been tested. Most assessments of sediment toxicity involve more than one species, although they are usually tested separately by adding a known number of specimens to the exposure chamber. When community response is examined, densities are not manipulated and animals are transferred from the field as intact assemblages or allowed to recruit as larvae from the water column in flow-through or field exposures.

Bioassays have been applied to material collected from contaminated field sites or to unpolluted sediment to which toxic materials have been added. Sediments from many contaminated field sites have now been examined, but bioassays with specific chemicals are not common. The intact ("solid phase") sediment is usually tested, and suspended or liquid sediment phases are sometimes examined, especially in relation to dredge material disposal. In contrast to the literature on the bioaccumulation of pollutants, relatively few reports concern relationships between sediment toxicity and the geochemistry of contaminants. The quantity of sediment and duration of exposure vary greatly: from $<<$ 1 L to $>$ 800 L and from 10 min to more than a year, respectively. Both static and flow-through seawater systems have been employed successfully. The simpler methods (i.e., acute mortality in static seawater systems with relatively small quantities of sediment) are commonly used in regulatory programs. Field validation of the ecological relevance of such systems is rare. The more logistically complicated methods (i.e., macrobenthic community response during long-term exposures) appear to provide a better simulation of natural benthic ecosystem.

## RESEARCH NEEDS AND ISSUES

### Test Species Selection

The primary criteria for test species selection should be the species' ecological importance and its relative sensitivity to sediment contamination. Laboratory bioassays and field surveys have shown that many species survive well in nature where sediment is acutely toxic to more sensitive species (19, 46, 47). It is possible to select indigenous, ecologically significant, and easily collected taxa that will survive in almost any sediment. Some of these taxa (e.g., *Macoma, Nereis, Glycinde*) are included on the EPA/USACE (1) list of recommended species for solid-phase bioassays. Such species may be appropriate for some sublethal response criteria, but they should be avoided for acute toxicity bioassays because of their tolerance to sediment contaminants.

The best guide for bioassay species selection lies in the wealth of data



available about the distribution of the macrobenthos near sites of contaminated sediment. On the basis of the extensive macrobenthic surveys that have been conducted in the Southern California Bight, Mearns and Word (48) identified nineteen species that were dominant in control or background areas and decreased in abundance with increasing proximity to sewage discharges. Twelve of the nineteen species were either phoxocephalid or ampeliscid amphipods. The distribution of amphipods in Puget Sound (19), the New York Bight (49), and many other coastal sites indicates that many amphipods are appropriately sensitive to sediment contaminants. This sensitivity has been confirmed in a variety of bioassays (10, 19, 30, 33, 46). The field distributions of many untested taxa also indicate promise as bioassay species, e.g., brittlestars (*Amphiodia*), polychaetes (*Sthenelanella*) and phoronids (*Phoronis*) (48).

Experimental comparison of the effects of contaminated sediment on different marine organisms is very relevant to test species selection. Shuba et al. (29) reported that the copepod *Acartia tonsa* was more sensitive than the copepod *Tigriopus californicus* to the liquid and suspended phases of Duwamish River, Washington, sediment. They also found that the isopod *Sphaeroma quadridentatum*, the mysid *Mysidopsis bahia*, the amphipod *Parahaustorius* sp., and larvae of the shrimp *Palaemonetes* spp. were more sensitive than adult *Palaemonetes* and the bivalves *Rangia cuneata* and *Mercenaria mercenaria* to the solid phase of several field sediments. Swartz et al. (18) found that the amphipod *Rhepoxynius abronius* and several cumaceans were more sensitive than two molluscs (*Macoma inquinata*, *Protothaca staminea*) or a polychaete (*Glycinde picta*) to sediment from the Houston Ship Channel and other field sites. Additional comparisons like these are desirable not just for the selection of test species, but also to elucidate factors controlling the distribution of different invertebrates along pollution gradients.

Two factors that are correlated with a species' sensitivity to sediment contaminants are its phylogenetic position and its relation to the substrate. For example, amphipods and other crustaceans are generally more sensitive than molluscs and polychaetes. Within phylogenetic groups, the infauna appear more sensitive than the epifaunal, demersal, or pelagic biota. Among the infauna, the most sensitive taxa are those that burrow beneath the sediment surface without extending tubes or body parts into overlying water, e.g., phoxocephalid amphipods. Such animals are greatly dependent on the quality of interstitial water and sediment particulates.

Shuba et al. (50) discussed several criteria for selection of test species and a rating system for comparing the suitability of several species. The criteria include whether the species: is indigenous to the disposal site or closely related to an indigenous species; is available through collecting or purchase; has a toxicological data base; can be maintained and cultured in the laboratory; is ecologically and economically important; has a wide geographic

tribution; and is compatible with other test species. Many species that may be appropriate for sediment bioassays do not meet these criteria simply because the focus of pollution ecology has historically been on water quality rather than sediment quality. Culture methods and toxicological data bases have not been developed for many infaunal animals. As these data are developed, they should include information about the effects of natural sediment variables (organic content, particle size distribution, interstitial water salinity, etc.) on test species. Analysis of sediment bioassay data should be able to discriminate effects of chemical contamination from those attributable to habitat alteration. Use of indigenous species promotes site specificity and allows comparison of sediment toxicity with the field distribution of the test species. However, indigenous species that are tolerant of sediment contaminants should not be selected.

### Selection of Response Criterion

Bioassays with mortality as an endpoint are usually not very sensitive to sediment contaminants, but these bioassays have the advantage of being more easily interpreted than sublethal tests (46). Such bioassays are included in the EPA/USACE Implementation Manual (1) and are widely applied in permit programs for ocean disposal. The key to the success of these acute, lethal bioassays lies in the selection of sensitive test species like phoxocephalid and ampeliscid amphipods.

Sublethal responses are generally more sensitive, but their application in permit programs is sometimes limited by the "So what?" syndrome. The ecological significance of induction of an enzyme system or changes in the rate of respiration is less obvious than mortality, genotoxicity, or reductions in population density and species richness. However, sublethal endpoints can indicate exposure to bioavailable contaminants in sediment, and the biological processes through which benthic organisms attempt to adapt to the exposure. Behavioral responses are particularly relevant. Oakden et al. (10) found that phoxocephalid amphipods avoided sediment contaminated with metals and sewage and concluded that behavioral avoidance might explain the absence of phoxocephalids near sewage outfalls. Pearson et al. (16) demonstrated that shallow burial and slower reburrowing of the clam *Protothaca staminea* in oiled sediment led to increased predation on the clam by Dungeness crabs (*Cancer magister*). They noted that this ecological effect would not have been predicted by standard toxicity tests.

Use of macrobenthic or meiobenthic assemblages has many advantages over use of one or a few test species. These designs are usually based on larval recruitment or collection of entire assemblages, and therefore no a priori species selection is required. Scaling of micro- and mesocosms with respect to size, duration, and ecological components generally provides a much better simulation of natural benthic ecosystems. These systems are highly



desirable research tools, but their application in routine regulatory programs is limited by logistical constraints.

Research is needed to compare the efficacy of different bioassay methods. Chapman et al. (3, 4, 5, 46, 47) compared several methods as part of a comprehensive survey of sediment toxicity in Puget Sound. These methods include acute lethality of an oligochaete, amphipod, polychaete, and fish; effects on the respiration of an oligochaete; success of oyster and fish larval development; chromosomal damage to fish gonad cells (anaphase aberration); and alterations in the population dynamics of a polychaete. They found that individual tests gave different toxic responses to sediment from the same station. Acute lethality was fairly rare and restricted to a few sensitive species like phoxocephalid amphipods. They believed that different tests might be sensitive to different contaminants. The anaphase aberration test for genotoxicity was very sensitive to sediment extracts. Despite differences in relative sensitivity there was a good correspondence among the methods in geographic trends of sediment toxicity. Chapman and Long (46) concluded that several bioassays with different response criteria should be used to evaluate toxicity.

Interlaboratory comparisons of bioassay methods are needed to establish confidence in the reproducibility of results. Five laboratories in the Puget Sound area have recently conducted phoxocephalid amphipod bioassays on seven different sediments collected from four field sites or contaminated artificially with three concentrations of cadmium (60). There was good agreement among the laboratories in the relative and absolute toxicity of the test sediments.

### Experimental Design

Test duration and size of the bioassay chamber vary greatly with the response criterion and test species (Table 1). Ten-day exposures seem appropriate for acute mortality tests as recommended by the EPA/USACE Implementation Manual (1, 22). Sublethal effects, especially behavioral changes, are evident usually in less than 10 d. Impacts on populations or communities depend on longer-term biological processes (recruitment, reproduction, growth, competition) that usually require several months or longer for completion. An exception is the bioassay developed recently by Tietjen and Lee (32) that measures impacts on the population dynamics of nematodes after 2 weeks' exposure to test sediment. Meiobenthic taxa like nematodes are rarely used in sediment bioassays or benthic pollution ecology in general. Because of their abundance, ecological significance, and short life histories, the meiobenthos offer experimental opportunities that should be exploited (32).

Differences between flow-through and static seawater systems may not be important for acute sublethal and lethal bioassays. Such tests generally do

attempt to simulate transport processes of bioturbation and physical mixing of sediment. Since molecular diffusion is a minor short-term mechanism of exchange of solutes between interstitial and overlying waters (51), contaminant concentrations remain essentially constant during acute bioassays. Static systems are not acceptable if an attempt is made to simulate the fate of contaminants.

Geochemical properties of sediments change during storage. Drying, freezing, and cold storage affect toxicity (52, 53, 54). Swartz et al. (22) recommended that sediment be stored at 4°C for no more than 5 d before the initiation of bioassays.

Effects of storage are only one manifestation of the lack of knowledge about geochemical changes in sediment in the laboratory. In particular, redox potential and partitioning of contaminants between interstitial water and particles are altered under laboratory conditions, but rarely monitored during bioassays. The ecological significance of bioassays depends on the generally untested assumption that contaminant bioavailability in the laboratory simulates conditions in natural benthic ecosystems. This uncertainty is especially serious for bioassays in which contaminants are experimentally added to sediment.

There are several applications of sediment bioassays in marine pollution ecology. As part of permitting programs, they are used to determine the acceptability of dredged materials and other wastes for ocean disposal (1). This involves a comparison of the toxicity of the waste material to a sediment that represents the disposal site or some other reference condition. It is possible to add complex wastes like sewage sludge to uncontaminated sediment and obtain an estimate of the relative toxicity of wastes from different sources (20). Unfortunately, measures of the toxicity of complex wastes cannot be easily related to specific chemicals. The question of causal factors can be directly examined by adding known quantities of specific chemicals to nontoxic sediment (25, 26, 59). Bioassay results are used to estimate $LC_{50}$s or related measures of safe concentrations. This provides at least a conceptual basis for sediment quality criteria. However, the success of this relatively new research area depends largely on the problem of relating toxicity to a measure of the bioavailable fraction of bulk sediment concentrations. Spatial distributions of sediment toxicity near disposal sites or urbanized embayments can be identified by bioassays of field samples collected at many stations in the area of interest (3, 4, 19, 21, 23, 27). Such surveys can efficiently locate "hot spots" where more comprehensive study is warranted. They can be applied in monitoring programs to detect unacceptable perturbations, e.g., beyond a dump site boundary; or in research projects where sediment toxicity can be compared with sediment contamination or the structure of benthic communities. Sediment bioassays are also used to address more fundamental questions about processes of degradation and recovery of contaminated benthic ecosystems (37, 45). This research usually involves field exposures or larger laboratory systems that attempt to simulate natural conditions accurately.



It is difficult to identify specific causes of toxicity associated with sediment collected from field sites. There are three general problems: (1) to discriminate effects of sediment chemical contaminants (metals, PCBs, etc.) from effects of natural sediment properties (organic carbon, grain size, etc.), (2) to discriminate effects of different chemical contaminants, and (3) to identify and quantify interactions between different contaminants and between contaminants and natural sediment properties. The first problem is important because organic enrichment and chemical contamination have different regulatory implications (20). The structure of benthic communities is greatly affected by the organic carbon content of sediment (62). In chemically contaminated benthic habitats such as the Palos Verdes Shelf, California, organic enrichment and anaerobic sediment conditions appear to have a greater anthropogenic influence on the benthos than chemical contaminants (21). If sediment bioassays are used to detect toxicity associated with chemical pollutants, it is necessary to demonstrate that the bioassay is not responding to other substrate alterations. The second problem arises because polluted marine habitats are often affected by many chemical contaminants. A method has recently been proposed to identify a toxicity "apparent effect threshold" (AET) for individual chemicals based on chemical and bioassay data for field-collected sediment samples (63). The AET is defined as the contaminant concentration above which significant sediment toxicity would always be expected. The AET method is most useful when sediment toxicity is associated primarily with single factors. The third problem, concerning synergistic or other interactions, has received little attention. Some method of quantifying these interactions must be developed before it will be possible to identify the joint toxic action of combinations of chemicals in sediment. Fortunately, some conceptual and mathematical models exist for describing interaction between chemicals dissolved in water (64, 65, 66). It may be possible to apply these models directly to interstitial water or to particle-bound contaminant concentrations after accounting for partitioning to the aqueous phase.

There are inherent limitations in the ability of acute laboratory bioassays to reflect the full range of ecological consequences of sediment contamination. Nonetheless, such bioassays are being used in hazard assessment protocols, often in the initial tier of biological evaluation. When bioassays determine acceptability for ocean disposal of waste material, their results should be applied in an environmentally protective manner. If there is any evidence for acute toxicity, ocean disposal should be denied or the waste material should be subjected to more comprehensive analyses. It may therefore be inappropriate to use normal statistical conventions to compare control and test material data. If a null hypothesis of no difference between control and test means is tested at the 95% significance level (alpha = 0.05),

there can be no more than a 5% chance of rejecting a true null hypothesis (type I error) (55). However, the probability of not rejecting a false null hypothesis (type II error) can be very large (as great as 95%). Normal statistical criteria therefore can permit the ocean discharge of waste materials that are in reality more toxic than the bioassay control. The solution to this problem is counterintuitive to most scientists, i.e., the significance level must be decreased rather than increased. The probability of a type II error (and the probability of discharging toxic wastes) would be greatly reduced if the null hypothesis of no difference between control and tests means is rejected at alpha values exceeding 0.50, i.e, if the probability of a type I error is 50% or more. Alternative statistical methods that directly define the change of a type II error could also be used.

### Field Validation

The ecological relevance of several bioassays has been tested by comparing the toxicity of sediment along a pollution gradient with biological communities or sediment contamination along the same gradient (10, 19, 21, 32, 47). Swartz et al. (19) found that phoxocephalid amphipods were absent from the industrialized waterways of Commencement Bay, Washington, where sediment was acutely toxic in laboratory bioassays to the phoxocephalid, *Rhepoxynius abronius*, but phoxocephalids were ubiquitous in the deeper portion of the bay where sediment was not toxic. Similarly, the survival of *R. abronius* in bioassays of sediment from the Palos Verdes Shelf, California, near the Los Angeles County sewage outfall, was significantly correlated with the spatial distribution of phoxocephalids, the structure of benthic communities, and sediment contamination (21). Oakden et al. (10) showed that phoxocephalids avoided contaminated sediment from the Palos Verdes Shelf. Tietjen and Lee (32) reported significant correlations between chemical contamination and the growth of nematode populations in sediment collected along a pollution gradient in the Hudson-Raritan estuarine area adjacent to the New York Bight.

The ecological relevance of marine mesocosms is dependent on the strategy of either capturing or precisely simulating a natural system. The mesocosms of the Marine Ecosystem Research Laboratory at the University of Rhode Island simulate both benthic and pelagic dynamics of Narragansett Bay (41-44). This simulation is greatly enhanced by the initial placement of intact sediment cores in the mesocosm and the addition of Narragansett Bay water to the mesocosm during experiments.

### Research Priorities

There are innumerable methodological problems that should be resolved. These include comparative study of species sensitivity and different response



criteria, development of better sediment storage methods, more realistic incorporation of sediment bioassays into hazard assessment protocols, and field validation of bioassay results. However, sediment bioassay methods are sufficiently advanced that research should now be directed toward more fundamental questions. Three research needs that have a high priority are (1) the relation between toxicity and the bioavailability of sediment contamination, (2) the importance of interactions between contaminants in determining sediment toxicity, and (3) the functional significance of sediment toxicity, particularly in relation to trophic dynamics and benthic-pelagic coupling. Bioassay methods that concern sublethal and lethal effects on individuals may present the best experimental approach to the first two of these research areas. Clearly, functional changes in benthic ecosystems should be addresses in larger-scale and longer-term experiments with micro- and mesocosms.

## REFERENCES


1. **Environmental Protection Agency/U.S. Army Corps of Engineers.** 1977. Ecological evaluation of proposed discharge of dredged material into ocean waters: Implementation manual for section 103 of PL 92-532. Environmental Effects Laboratory, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
2. **Lee, R. F., S. C. Singer, K. R. Tenore, W. S. Gardner and R. M. Philpot.** 1979. Detoxification system in polychaete worms: Importance in the degradation of sediment hydrocarbons. In W. B. Vernberg, A. Calabrese, F. P. Thurberg and F. J. Vernberg, eds., Marine Pollution: Functional Responses. Academic Press, New York, pp. 23-37.
3. **Chapman, P. M., G. A. Vigers, M. A. Farrell, R. N. Dexter, E. A. Quinlan, R. M. Kocan and M. Landolt.** 1982. Survey of biological effects of toxicants upon Puget Sound biota. I. Broad-scale toxicity survey. NOAA Technical Memorandum OMPA-25, Boulder, CO.
4. **Chapman, P. M., D. R. Munday, J. Morgan, R. Fink, R. M. Kocan, M. L. Landolt and R. N. Dexter.** 1983. Survey of biological effects of toxicants upon Puget Sound biota. II. Tests of reproductive impairment. NOAA Technical Report NOS 102 OMS 1, Rockville, MD.
5. **Chapman, P. M. and J. D. Morgan.** 1983. Sediment bioassays with oyster larvae. *Bull. Environ. Contam. Toxicol.* 31:438-444.
6. **DeCoursey, P. J. and W. B. Vernberg.** 1975. The effect of dredging in a polluted estuary on the physiology of larval zooplankton. *Water Res.* 9:149-154.
7. **Hoss, D. E., L. C. Coston and W. E. Schaaf.** 1974. Effects of sea water extracts of sediments from Charleston Harbor, S. C., on larval estuarine fishes. *Estuar. Coastal Mar. Sci.* 2:323-328.
8. **Kehoe, D. M.** 1983. Effects of Grays Harbor estuary sediment on the osmoregulatory ability of coho salmon smolts (*Oncorhynchus kisutch*). *Bull. Environ. Contam. Toxicol.* 30:522-529.
9. **Chang, B. D. and C. D. Levings.** 1976. Laboratory experiment on the effects of ocean dumping on benthic invertebrates. I. Choice tests with solid wastes. Fisheries and Marine Service Technical Report No. 637, West Vancouver, B.C.
10. **Oakden, J. M., J. S. Oliver and A. R. Flegal.** 1984. Behavioral responses of a phoxocephalid amphipod to organic enrichment and trace metals in sediment. *Mar. Ecol. Progr. Series* 14:253-257.
11. **Mohlenberg, F. and T. Kiorboe.** 1983. Burrowing and avoidance behaviour in marine organisms exposed to pesticide-contaminated sediment. *Mar. Pollut. Bull.* 14:57-60.
12. **Pearson, W. H., D. L. Woodruff, P. C. Sugarman and B. O. Olla.** 1984. The burrowing behavior of sand lance, *Ammodytes hexapterus:* Effects of oil-contaminated sediment. *Mar. Environ. Res.* 11:17-32.

13. **Olla, B. L. and A. J. Bejda.** 1983. Effects of oiled sediment on the burrowing behaviour of the hard clam, *Mercenaria mercenaria. Mar. Environ. Res.* 9:183-193.
14. **Phelps, H. L., J. T. Hardy, W. H. Pearson and C. W. Apts.** 1983. Clam burrowing behaviour: Inhibition by copper-enriched sediment. *Mar. Pollut. Bull.* 14:452-455.
15. **Rubinstein, N.I.** 1979. A benthic bioassay using time-lapse photography to measure the effect of toxicants on the feeding behavior of lugworms (Polychaeta: Arenicolidae). In W. B. Vernberg, A. Calabrese, F. P. Thurberg and F. J. Vernberg, eds., *Marine Pollution: Functional Responses.* Academic Press, New York, pp. 341-351.
16. **Pearson, W.H., D. L. Woodruff, P. C. Sugarman and B. L. Olla.** 1981. Effects of oiled sediment on predation on the littleneck clam, *Protothaca staminea,* by the Dungeness crab, *Cancer magister. Estuar. Coastal Shelf Sci.* 13:445-454.
17. **Nimmo, D. R., T. L. Hamaker, E. Matthews and W. T. Young.** 1982. The long-term effects of suspended particulates on survival and reproduction of the mysid shrimp, *Mysidopsis bahia,* in the laboratory. In G. F. Mayer, ed., *Ecological Stress and the New York Bight: Science and Management.* Estuarine Research Federation, Columbia, SC, pp. 413-422.
18. **Swartz, R. C., W. A. DeBen and F. A. Cole.** 1979. A bioassay for toxicity of sediment to marine macrobenthos. *J. Water Pollut. Control Fed.* 51:944-950.
19. **Swartz, R. C., W. A. DeBen, K. A. Sercu and J. O. Lamberson.** 1982. Sediment toxicity and the distribution of amphipods in Commencement Bay, Washington, USA. *Mar. Pollut. Bull.* 13:359-364.
20. **Swartz, R. C., D. W. Schults, G. R. Ditsworth and W. A. DeBen.** 1984.Toxicity of sewage sludge to *Rhepoxynius abronius,* a marine benthic amphipod. *Arch. Environ. Contam. Toxicol.* 13:207-216.
21. **Swartz, R. C., D. W. Schults, G. R. Ditsworth, W. A. DeBen and F. A. Cole.** 1985. Sediment toxicity, contamination, and macrobenthic communities near a large sewage outfall. In T. P. Boyle, ed., *Validation and Predictability of Laboratory Methods for Assessing the Fate and Effects of Contaminants in Aquatic Ecosystems.* ASTM STP 865. American Society for Testing and Materials, Philadelphia, PA, pp. 152-175.
22. **Swartz, R. C., W. A. DeBen, J. K. P. Jones, J. O. Lamberson and F. A. Cole.** 1985. Phoxocephalid amphipod bioassay for marine sediment toxicity. In R. D. Cardwell, R. Purdy and R. C. Bahner, eds., *Aquatic Toxicology and Hazard Assessment: Seventh Symposium.* ASTM STP 854. American Society for Testing and Materials, Philadelphia, PA, pp. 284-307.
23. **Tsai, C., J. Welch, K. Chang, J. Shaefer and L. E. Cronin.** 1979. Bioassay of Baltimore Harbor sediments. *Estuaries* 2:141-153.
24. **Rubinstein, N. I., W. T. Gilliam and N. R. Gregory.** 1983. Evaluation of three fish species as bioassay organisms for dredged material testing. EPA-600/X-83-062. U.S. Environmental Protection Agency, Gulf Breeze, FL.
25. **McLeese, D. W. and C. D. Metcalfe.** 1980. Toxicities of eight organochlorine compounds in sediment and seawater *Crangon septemspinosa. Bull. Environ. Contam. Toxicol.* 25:921-928.
26. **McLeese, D. W., L. E. Burridge and J. Van Dinter.** 1982. Toxicities of five organochlorine compounds in water and sediment to *Nereis virens. Bull. Environ. Contam. Toxicol.* 28:216-210.
27. **Alden, R. W., III and R. J. Young, Jr.** 1982. Open ocean disposal of materials dredged from a highly industrialized estuary: An evaluation of potential lethal effects. *Arch. Environ. Contam. Toxicol.* 11:567-576.
28. **Lee, G. F. and G. M. Mariani.** 1976. Evaluation of the significance of waterway sediment-associated contaminants on water quality at the dredged material disposal site. In F. L. Mayer and J. L. Hamelink, eds., *Aquatic Toxicology and Hazard Evaluation.* ASTM STP 634. American Society for Testing and Materials, Philadelphia, PA, pp. 196-213.
29. **Shuba, P. J., H. E. Tatem and J. H. Carroll.** 1978. Biological assessment methods to predict the impact of open-water disposal of dredged material. Technical Report D-78-50. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
30. **Scott, K. J., P. P. Yevich and W. S. Boothman.** Toxicological methods using the benthic amphipod *Ampelisca abdita* Mills. (Submitted to *Aquat. Toxicol.*)



31. **Peddicord, R. K.** 1980. Direct effects of suspended sediments on aquatic organisms. In R. A. Baker, ed., *Contaminants and Sediments,* Vol. 1. Ann Arbor Science Publishers, Ann Arbor, MI, pp. 501-536.
32. **Tietjen, J. H. and J. J. Lee.** 1984. The use of free-living nematodes as a bioassay for estuarine sediments. *Mar. Environ. Res.* 11:233-251.
33. **MacKenzie, C. L., Jr., D. Radosh and R. N. Reid.** 1983. Reduced numbers of early stages of Mollusca and Amphipoda on experimental sediments containing sewage sludge. *Coastal Ocean Pollution Assessment News* 3:7-8.
34. **Hansen, D. J. and M. E. Tagatz.** 1980. A laboratory test for assessing impacts of substances on developing communities of benthic estuarine organisms. In J. G. Eaton, P. R. Parrish and A. C. Hendricks, eds., *Aquatic Toxicology.* ASTM STP 707. American Society for Testing and Materials, Philadelphia, PA, pp. 40-57.
35. **Hansen, D. J.** 1974. Aroclor 1254: Effect on composition of developing estuarine animal communities in the laboratory. *Mar. Sci.* 18:19-33.
36. **Tagatz, M. E. and M. Tobia.** 1978. Effects of barite ($BaSO_4$) on development of estuarine communities. *Estuar. Coastal Mar. Sci.* 7:401-407.
37. **Vanderhorst, J. R., J. W. Blaylock, P. Wilkinson, M. Wilkinson and G. Fellingham.** 1980. Effects of experimental oiling on recovery of Strait of Juan De Fuca intertidal habitats. EPA-600/7-81-008. U.S. Environmental Protection Agency, Washington, DC.
38. **Young, D. K. and M. W. Young.** 1978. Regulation of species densities of seagrass-associated macrobenthos: Evidence from field experiment in the Indian River estuary, Florida. *Mar. Res.* 36:569-593.
39. **Eleftheriou, A., D. C. Moore, D. J. Basford and M. R. Robertson.** 1982. Underwater experiments on the effects of sewage sludge on a marine ecosystem. *Neth. Sea Res.* 16:465-473.
40. **Perez, K. T.** 1983. Environmental assessment of a phthalate ester, di(2-ethylhexyl) phthalate (DEHP), derived from a marine microcosm. In W. E. Bishop, R. D. Cardwell and B. B. Heidolph, eds., *Aquatic Toxicology and Hazard Assessment.* ASTM STP 801. American Society for Testing and Materials, Philadelphia, PA, pp. 180-191.
41. **Elmgren, R., G. A. Vargo, J. F. Grassle, J. P. Grassle, D. R. Heinle, G. Langlois and S. L. Vargo.** 1980. Trophic interactions in experimental marine ecosystems perturbed by oil. In J. P. Giesy, Jr., ed., *Microcosms in Ecological Research.* U.S. Department of Energy, Symposium Series 52, Washington, DC, pp. 779-800.
42. **Grassle, J. F., R. Elmgren and J. P. Grassle.** 1981. Response of benthic communities in MERL experimental ecosystems to low level chronic additions of No. 2 fuel oil. *Mar. Environ. Res.* 4:279-297.
43. **Oviatt, C., J. Frithsen, J. Gearing and P. Gearing.** 1982. Low chronic additions of No. 2 fuel oil: Chemical behavior, biological impact and recovery in a simulated estuarine environment. *Mar. Ecol. Prog. Ser.* 9:121-136.
44. **Oviatt, C. A., M. E. Q. Pilson, S. W. Nixon, J. B. Frithsen, D. T. Rudnick, J. R. Kelly, J. F. Grassle and J. P. Grassle.** 1984. Recovery of a polluted estuarine system: A mesocosm experiment. *Mar. Ecol. Prog. Ser.* 16:203-217.
45. **Kuiper, J., P. DeWilde and W. Wolff.** 1984. Effects of an oil spill in outdoor model tidal flat ecosystems. *Mar. Pollut. Bull.* 15:102-106.
46. **Chapman, P. M. and E. R. Long.** 1983. The use of bioassays as part of a comprehensive approach to marine pollution assessment. *Mar. Pollut. Bull.* 14:81-84.
47. **Chapman, P. M., R. N. Dexter, R. M. Kocan and E. R. Long.** 1985. An overview of biological effects testing in Puget Sound, Washington: Methods, results and implications, in R. D. Cardwell, R. Purdy and R. C. Bahner, eds., *Aquatic Toxicology and Hazard Assessment*: Seventh Symposium. ASTM STP 854. American Society for Testing and Materials, Philadelphia, PA, pp. 344-363.
48. **Mearns, A. J. and J. Q. Word.** 1982. Forecasting effects of sewage solids on marine benthic communities. In G. F. Mayer, ed., *Ecological Stress and the New York Bight: Science and Management.* Estuarine Research Federation, Columbia, SC, pp. 495-512.
49. **National Marine Fisheries Service.** 1972. The effects of waste disposal in the New York Bight. Final Report, Section 2: Benthic studies. NTIS Rept. No. AD739532. Sandy Hook Sports Fisheries Mar. Lab., Highland, NJ.

50. **Shuba, P. J., S. R. Petrocelli and R. E. Bentley.** 1981. Considerations in selecting bioassay organisms for determining the potential environmental impact of dredged material. Technical Report EL-81-8. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
51. **Christensen, J. P., A. H. Devol and W. M. Smethie, Jr.** 1984. Biological enhancement of solute exchange between sediments and bottom water on the Washington continental shelf. *Continental Shelf Research* 3:9-23.
52. **Lee, G. F. and R. H. Plumb.** 1974. Literature review on research study for the development of dredged material disposal criteria. Contract Report D-74-1. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
53. **Lee, G. F. and R. A. Jones.** 1982. Dredged material evaluations: Correlation between chemical and biological evaluation procedures. *J. Water Pollut. Control Fed.* 54:406.
54. **Dillon, T.** 1983. The effects of storage temperature and time on sediment toxicity. Poster presented at Fourth Annual SETAC Meeting, Arlington, VA. U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
55. **Sokal, R. R. and F. J. Rohlf.** 1981. *Biometry*, 2nd ed. W. H. Freeman and Company, San Francisco, CA.
56. **Schiewe, M. H., E. G. Hawk, D. I. Actor and M. M. Krahn.** 1985. Use of a bacterial bioluminescence assay to test toxicity of contaminated marine sediments. *Can. J. Fish. Aquat. Sci.* 42:1244-1248.
57. **Landolt, M. L. and R. M. Kocan.** 1984. Lethal and sublethal effects of marine sediment extracts on fish cells and chromosomes. *Helgolander Meersunters.* 37:479-491.
58. **Hargis, W. J., Jr., M. H. Roberts, Jr. and D. E. Zwerner.** 1984. Effects of contaminated sediments and sediment-exposed effluent water on an estuarine fish: acute toxicity. *Mar. Environ. Res.* 14:337-354.
59. **Swartz, R. C., G. R. Ditsworth, D. W. Schults and J. O. Lamberson.** 1986. Sediment toxicity to a marine infaunal amphipod: cadmium and its interaction with sewage sludge. *Mar. Environ. Res.* 18 (in press).
60. **Mearns, A. J., R. C. Swartz, J. M. Cummins, P. A. Dinnel, P. Plesha and P. M. Chapman.** 1986. Inter-laboratory comparison of a sediment toxicity test using the marine amphipod, *Rhepoxynius abronius*. *Mar. Environ. Res.* 18 (in press).
61. **Chapman, P. M. and R.Fink.** 1984. Effects of Puget Sound sediments and their elutriates on the life cycle of *Capitella capitata*. *Bull. Environ. Contam. Toxicol.* 33:451-459.
62. **Pearson, T. H. and R. Rosenberg.** 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanogr. Mar. Biol. Ann. Rev.* 16:229-311.
63. **Tetra Tech, Inc.** 1985. Commencement Bay nearshore/tideflats remedial investigation. Washington State Dept. Ecology, Olympia, WA. TC-3752, Vol. 1, section 4.2.
64. **Sprague, J. B.** 1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. *Water Res.* 4:3-32.
65. **Voyer, R. A. and J. F. Heltshe.** 1984. Factor interactions and aquatic toxicity testing. *Water Res.* 18:441-447.
66. **Christensen, E. R. and C. Y. Chen.** 1985. A general noninteractive multiple toxicity model including probit, logit, and Weibull transformations. *Biometrics* 41:711-725.



**NOTICE**

**This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.**

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.